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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

		Applicatio	n No.	No. Applicant(s)				
		10/554,030)	HAN ET AL.				
	Office Action Summary	Examiner		Art Unit				
		Michael C.	Wilson	1632				
The MAILING DATE of this communication appears on the cover sheet with the correspondence address Period for Reply								
A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION. - Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication. - If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication. - Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).								
Status								
2a) <u></u> 3) <u></u>	Responsive to communication(s) filed on This action is FINAL . 2b) Th Since this application is in condition for allow closed in accordance with the practice under	nis action is no vance except f	or formal matters, pro		e merits is			
Disposition of Claims								
5)□ 6)⊠ 7)□ 8)□ Applicatio	Claim(s) 1-17 is/are pending in the application is also of the above claim(s) is/are withdrays claim(s) is/are allowed. Claim(s) 1-17 is/are rejected. Claim(s) is/are objected to. Claim(s) are subject to restriction and/or Papers	rawn from con						
 9) The specification is objected to by the Examiner. 10) The drawing(s) filed on is/are: a) accepted or b) objected to by the Examiner. Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a). Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d). 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152. 								
Priority u	nder 35 U.S.C. § 119							
 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f). a) All b) Some * c) None of: 1. Certified copies of the priority documents have been received. 2. Certified copies of the priority documents have been received in Application No 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)). * See the attached detailed Office action for a list of the certified copies not received. 								
2) Notice 3) Inform	e of References Cited (PTO-892) of Draftsperson's Patent Drawing Review (PTO-948) nation Disclosure Statement(s) (PTO/SB/08) No(s)/Mail Date 10-20-05.	:	4)	te				

DETAILED ACTION

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-17 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of making a transgenic chicken comprising isolating PGCs from a chicken embryonic gonad, transferring a foreign gene to said PGCs, injecting the transfected PGCs into a recipient chicken embryo and incubating an egg containing said recipient chicken embryo until hatch whereby a transgenic chicken is obtained, does not reasonably provide enablement for a method of preparing a transgenic chicken by transfecting PGCs, culturing the transfected PGCs in vitro for at least 5 days and injecting the cultured, transfected PGCs into a recipient chicken embryo such that a transgenic chicken is obtained or making a germline chimera as claimed in any avian species other than chicken. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Stable transfection of PGCs in culture and making a transgenic germline chimeric avian

Claim 3 requires transferring a foreign gene into avian PGCs, culturing the transformed PGCs in vitro for at least 5 days, injecting the cultured PGCs into a

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recipient embryo and incubating and hatching an egg containing said recipient embryo, whereby the transgenic avian is produced. Claims 15-17 are dependent upon claim 3.

Vick (Proc. R. Soc. Lond., 1993, Vol. 251, pg 179-182) isolated stage XI PGCs from chickens, transduced them with retrovirus, and immediately injected them into the vasculature of Stage 15 chick embryos to obtain germline transmission. Vick did not teach how to stably culture transfected PGCs for at least 5 days such that a chimeric chicken that passed a transgene on to its offspring was obtained.

Allioli (Developmental Biol., 1994, Vol. 165, pg 30-37) expressed exogenous DNA in PGCs transfected with a retroviral vector and cultured for 2 days *in vitro* (pg 36, col. 1, lines 1-5). Allioli did not teach the PGCs were transfected and then cultured for at least 5 days or that the transfected PGCs were able to produce chimeric birds expressing exogenous protein.

Thoroval (Transgenic Research, 1995, Vol. 4, pg 369-376) injected retroviral vectors into the subgerminal cavity of an avian embryo in a freshly laid egg to obtain germline transmission of a transgene. Thoroval did not transfect PGCs in vitro or culture transfected PGCs for at least 5 days that were able to produce chimeric birds expressing exogenous protein.

Chang (Cell Biology International, 1995, Vol. 19, No. 6, pg 143-149) isolated the germinal ridge of day 5 chick embryos (stage 27-28) and cultured the cells for 5 days (pg 143, "Preparation of germinal ridge and culture of stroma cells"; pg 146, Fig. 2 and caption for Fig. 2). The culture was maintained for 10 days (pg 145, col. 1, last 4 lines). The germinal ridge cells were cultured in media comprising FBS, LIF, IGF and FGF-b

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(pg 144, col. 1, 1st full ¶). The germinal ridge cells comprised stromal cells (pg 144, line 6) (and PGCs (last sentence on pg 144: "The feeder layer derived from GRs must contain intrinsic PGCs")); the germinal ridge stromal cells are a "gonadal stroma feeder cell layer" as in claim 12.

Naito (March 31 - April 5, 1996, 6th International Symposium on avian endocrinology, "Expression of exogenous DNA in embryonic gonads by transferring primordial germ cells transfected in vitro", pg 69-73) isolated PGCs from the blood of a chicken embryo, transfected the chicken PGCs with a transgene *in vitro*, transferred the PGCs to a recipient chick embryo, obtained a chimeric chicken and obtained expression of the transgene in gonads of the chimeric chicken. Naito did not teach the <u>transfected</u> PGC genotype or phenotype was passed on to its offspring because Naito did not teach making offspring. In fact, Naito (1998, J. Reproduction and Fertility, Vol. 113, pg 137-143) later determined the transgene was <u>lost</u> during embryonic development because it was episomal (see abstract). Therefore, Naito 1996 did not teach how to stably transfect PGCs, make a chimeric chicken that passed a transgene on to its offspring using transfected PGCs or express the transgene in an egg.

Pain (Development, 1996, Vol. 122, pg 2339-2348) obtained ES cells from Stage X embryos within a mixed population of PGCs and cells that provide germline and somatic cell transmission. Pain taught marker proteins found on the mixed population of cells (pg 2345, col. 2). Pain did not teach how to stably transfect PGCs for at least 5 days or how to make a germline chimeric chicken that passed a transgene on to its offspring using transfected PGCs maintained in culture for at least 5 days.

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Park (Mol. Reproduction and Development, 2000, Vol. 56, pg 475-482) isolated PGCs from the germinal ridge of stage 28 embryos, culturing the cells in FBS, SCF, LIF, bFGF, IL-11 and IFG (pg 476, paragraph bridging col. 1-2). The cells were cultured for up to ten passages and proliferated over a period of 4 months (one month) (pg 477, col. 1, last 7 lines). The cells expressed the SSEA-1 epitope "after 4 passages (one month)" (pg 477, col. 2, first full paragraph). The cells were injected into the subgerminal cavity of recipient embryos (pg 477, col. 1, "Production of Somatic chimeras"). One of the 5 chimeras showed chimerism in all tissues sampled including the gonad; two hatched chicks showed contribution to the gonad (pg 479, col. 2, lines 1-8).

Zandong (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85) isolated gonad PGCs from 5.5-day-old chicken embryos and cultured them on gonad stroma cells with addition of 5 ng/ml SCF and 10 ng/ml LIF. They proliferated for 10 days in culture. The colony appeared to be positive after PAS staining. A 1.2 kb fragment of the chicken ovalbumin gene promoter sequence cloned by PCR was used to replaced the promoter of vector pEGFP (Clontech). Lysozyme gene and ovalbumin gene's first and second exons including signal sequence also have been cloned and inserted into the multiple clone sites upstream to the EGFP gene in modified pEGFP. Cultured CEFs were transfected with the constructed vector by lipofectin (Gibco) and showed faint fluorescence after 24 h culture. Cultured CEFs (DMEM/F12+10%FBS) were transfected with 4µg plasmid pEGFP-C1 (Clontech) by lipofectin (Gibco), followed by electroporation under 200 voltage. The pEGFP-C1 mixed with lipofectin (1:2.5) were microinjected into chicken blastoderms (stage X) *in vivo*, followed by electroporation

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under 10 v, then the eggs were hatched for 72 h. GFP expression was examined under inverted fluorescent microscope. Transfection efficiency was improved by lipofection and electroporation. The same result was observed in early chicken embryos.

Zandong suggested performing further research focused on transfecting PGCs with the constructed vector and subsequently producing transgenic chickens.

Kim (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85) taught various attempts have been made to produce germline chimeras by the transfer of PGCs into the host embryos. Due to a limited number of PGCs, culture of PGCs isolated from the embryonic blood has been found to be difficult. However, Kim showed that a larger number of PGCs could be collected from developing gonads and successfully cultured in vitro. Kim demonstrated previously that transfer of cultured gonadal PGCs (gPGCs) to recipient embryos could produce germline chimeric chickens. Although gPGCs have been used in the production of germline chimera, efficiency has not been satisfactory. In the present study, Kim improved efficiency of germline chimerism by transfer of gPGCs cultured in vitro. Transfer of cultured gPGCs from Korean Ogol Chicken (KOC) to White Leghorn (WL) produced germline chimeric chickens. Gonadal PGCs were isolated from KOC embryonic gonads at stage 27 (5.5-day-old) and cultured in vitro for 10 days. Blood was withdrawn from the recipient via the dorsal aorta prior to transfusion. Approximately 200 cultured gPGCs were injected into the bloodstream through the dorsal aorta of stage 13–14 (2-day-old) recipient embryos. The recipient embryos were incubated until hatching. The recipients were mated with KOC. Donorderived offsprings were determined as germline chimeric chickens based on their

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feather color. From the hatched recipient chickens, 65 males and 88 females were brought up to maturity. These WLs (KOC) were mated with KOCs, resulting in 357 germline chimeric offsprings. A total of progenies were identified as germline chimeras by black feather color of their progenies. At 4–22 weeks of test period, the frequency of germline transmission of donor gPGCs were 1.0_56.0% (25.6% on average) for 14 chimeras. Thus, the donor gPGCs could be normally developed and differentiated into ova and spermatozoa in chimeric embryos and adult chickens, despite being surrounded by genetically different somatic cells.

Petitte (J. Poultry Sci., 4th quarter of 2002, Vol. 39, No. 4, pg 205-228) discusses cells of the avian germline and summarizes strategies for making transgenic chickens.

Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539) isolated PGCs from gonads of stage 28 chicken embryos and cultured them on gonadal stromal feeder cells with bovine serum, SCF, LIF, bFGF, IL-11 and IGF for two months (pg 1532, 2.1). The PGCs stained with SSEA-1 (pg 1533, 2.2). The PGCs were injected into the blood vessel of a recipient embryo (pg 1533, 2.3). Four individuals were germline chimeras (pg 1536, 3.3).

Ivarie (Trends in Biotechnology, Jan. 2003, Vol. 21, pg 14-19) taught that because of the complex process by which a bird makes and lays eggs, transgenic procedures for birds have lagged far behind those of other organisms. Ivarie cites Pain who taught long-term culture of non-transfected, blastodermal cells that provided germline transmission; however, no transgenic birds have been made using ES cells or PGCs transfected in culture. The biggest obstacle to overcome in making transgenic

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birds using transfected PGCs is the loss of germline competence during culture of transfected PGCs (pg 14, col. 2, 3rd full ¶, 1st sentence; pg 17, col. 1, 2nd full ¶, last two sentences; pg 17, sentence bridging col. 1-2; pg 17, col. 2, last sentence). Thus, a transgenic avian could not be made by transfecting PGCs and maintaining the transfected PGCs in culture.

This is confirmed by Naito (Animal Sci. J., June 2003, Vol. 74, pg 157-168) who summarized the manipulation of PGCs (pg 163). The art as of 2003 did not teach how to transfect PGCs, inject them into a recipient embryo and obtain a chimeric avian expressing the transgene (see the first paragraph of "Manipulation of primordial germ cells"). The art did not teach how to maintain transfected PGCs so they express a transgene while maintaining their ability to contribute to the germline after being injected into recipient embryos.

Overall, no transgenic birds capable of passing a transgene off to their offspring have been made using PGCs transfected in culture as claimed as supported by Ivarie (2003), Naito (2003) and the hurdle described in Naito (1998).

The specification states a foreign gene can be introduced by liposome-mediated transfection (pg 8, lines 17-22). The specification states the foreign gene can be an antibiotic-resistance gene (pg 9, lines 8-18).

The examples do not teach how to overcome the unpredictability in the art by teaching how to stably transfecting PGCs and maintaining the transgene for at least 5 days in culture. The examples do not teach injecting PGCs transfected with a transgene and maintained in culture for at least 5 days into a recipient embryo and

obtaining a germline chimeric avian that passes the transgene on to its offspring. In particular, the specification does not provide any guidance for one of skill to overcome the hurdle described by Naito 1998 so that stable transfection of the PGCs, incorporation of the transgene into the genome of the PGCs, or passage of the transfected PGC phenotype to the offspring occurs. Without such guidance it would require one of skill undue experimentation to stably transfect the cells of the invention and maintain them for at least 5 days such that a germline chimeric avian that carries the transgene is obtained. Accordingly, the specification does not enable transfecting PGCs cells, maintaining them for at least 5 days in culture and using them to make germline chimeric avians as claimed.

The breadth of avian

Upon overcoming the rejection above, the specification does not enable obtaining any species of avian PGCs or making any species of chimeric avian as broadly claimed in claims 1-3. The number of species within the genus of avian is immense compared to the one species of chickens described by applicants (see table of Bird Classification/Families of the Eastern US Birds). The specification does not correlate the structure of chicken PGCs to any other avian species and does not correlate the stages of chick embryos to other avian species. Without such guidance and in view of the absence of transgenic avian species in the art or the specification, it would have required one of skill undue experimentation to determine how to make a transgenic avian in species other than chickens. In a parallel example, the ability to make transgenic rats was elusive for 15 years after scientists first made transgenic mice

despite the similarities in mice and rat embryos and reproductive systems (Proudman, 2001, "The quest for transgenic poultry: birds are not mice with feathers" Biotechnology in Animal Husbandry, Vol. 5, Kluwer Academic Publishers, pg 283-299; pg 284, lines 1-6). 15 years of research to determine how to achieve germline transmission in avian species other than chickens is undue. Applicants have not provided the blaze marks for one of skill to determine which of the numerous methods of making transgenic chickens will be successful in other avian species. Mizuarai (Biochemical and Biophysical Res. Comm. Aug 24, 2001, Vol. 286, pg 456-463) taught making a transgenic quail using a VSV-G vector injected into embryos. The method described by Mizuarai did not require transfecting quail PGCs in culture. It would have required those of skill to think outside of the realm of transgenic chickens to obtain quail PGCs, which is more than "routine experimentation" or trial and error. Therefore, the claims should be limited to chicken cells and producing chimeric chickens.

Indefiniteness

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is indefinite because it is unclear how the method improves germline transmission efficiency of PGCs.

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Claims 2 and 3 are indefinite because the metes and bounds of "improved germline transmission efficiency" cannot be determined.

Claims 2 and 3 are indefinite because "the improved germline transmission efficiency" lacks antecedent basis.

Claim 3 is indefinite because "said transformed PGCs" in step c) lacks antecedent basis.

Claim 3 is indefinite because "said culture PGCs" in step d) lacks antecedent basis.

Claim 3 is indefinite because it does not clearly indicate the structural or functional feature of the chimeric avian that is improved. As written, incubating and hatching the egg is all that is require to prepare a transgenic avian. The phrase "improved germline transmission efficiency" in the preamble is inadequate to indicate the structural or functional feature of the chimeric avian that is improved.

Claim 5 is indefinite because it does not further limit claims 1-3. Claims 1-3 already require isolating the PGCs from embryonic gonad.

Claims 6-8 and 11-13 are indefinite because "said in vitro culture" lacks antecedent basis.

Claim 14 is indefinite because "said injecting" lacks antecedent basis in claim 1.

Claim 17 is indefinite because "PGCs exhibiting the antibiotic resistance property" lacks antecedent basis. It cannot be determined how the additional steps in 17 correlate to the steps in claim 3.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4, 5 and 8-14 are rejected under 35 U.S.C. 102(b) as being anticipated by Chang (Cell Biology International, 1997, Vol. 21, No. 8, pg 495-499).

Chang (1997) isolated germinal ridge stromal cells (GRSC) from day 5 (stage 27-28) embryos and cultured the cells for 5 days in media containing IGF, FGF and LIF.

The germinal ridge is an "embryonic gonad" as in claims 1, 2 and 5. Chang (1997) injected the 5-day PGC culture into recipient embryos via the dorsal aorta (pg 496, col. 1, "Injection of cultured PGCs into recipient embryos"). The gPGCs provided germline transmission (pg 496, "Preparation and culture of gPGCs"; pg 497, Fig. 1, "Progeny of germline chimeric chickens"). The germinal ridge cells were cultured in media comprising FBS (fetal bovine serum), LIF, IGF and FGF-b (pg 496, col. 1, "Preparation and culture of gPGCs), which meets the limitations of claims 8-11. The adhesive cells of the 5-day GRSC culture (last sentence of "Preparation and culture of gPGCs") are a "gonadal stroma feeder cell layer" as in claim 12 because they were isolated from the gonadal cells. The PGCs of Chang (1997) inherently express the stage specific embryonic antigen-1 (SSEA-1) as in claim 13 because they were capable of making germline chimeras.

Claims 1, 2 and 4-13 are rejected under 35 U.S.C. 102(b) as being anticipated by Park (Mol. Reproduction and Development, 2000, Vol. 56, pg 475-482).

Park isolated PGCs from the germinal ridge of stage 28 embryos and cultured the cells in FBS, SCF, LIF, bFGF, IL-11 and IFG (pg 476, paragraph bridging col. 1-2). The cells were cultured for up to ten passages and proliferated over a period of 4 months (one month) (pg 477, col. 1, last 7 lines). The cells expressed the SSEA-1 epitope "after 4 passages (one month)" (pg 477, col. 2, first full paragraph). The cells were injected into the subgerminal cavity of recipient embryos (pg 477, col. 1, "Production of Somatic chimeras"). One of the 5 chimeras showed chimerism in all tissues sampled including the gonad; two hatched chicks showed contribution to the gonad (pg 479, col. 2, lines 1-8).

Claims 1, 2, 4-7, 13 and 14 are rejected under 35 U.S.C. 102(b) as being anticipated by Kim (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001).

Kim produced germline chimeric chickens by transfer of cultured gPGCs from Korean Ogol Chicken (KOC) to White Leghorn (WL). Gonadal PGCs were isolated from KOC embryonic gonads at stage 27 (5.5-day-old) and cultured *in vitro* for 10 days. Approximately 200 cultured gPGCs were injected into the bloodstream through the dorsal aorta of stage 13–14 (2-day-old) recipient embryos. The recipient embryos were incubated until hatching. The recipients were mated with KOC. Donor-derived offsprings were determined as germline chimeric chickens based on their feather color.

From the hatched recipient chickens, 65 males and 88 females were brought up to maturity. These WLs (KOC) were mated with KOCs, resulting in 357 germline chimeric offsprings. A total of progenies were identified as germline chimeras by black feather color of their progenies. At 4–22 weeks of test period, the frequency of germline transmission of donor gPGCs were 1.0_56.0% (25.6% on average) for 14 chimeras. Thus, the donor gPGCs could be normally developed and differentiated into ova and spermatozoa in chimeric embryos and adult chickens, despite being surrounded by genetically different somatic cells. Claim 13 is included because the cells in culture for 10 days and injected into recipient embryos inherently express SSEA-1 because they were PGCs capable of contributing to the germline.

Claims 1, 4-10 and 13 are rejected under 35 U.S.C. 102(b) as being anticipated by Zandong (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001).

Zandong isolated gonad PGCs from 5.5-day-old chicken embryos and cultured them on gonad stroma cells with addition of 5 ng/ml SCF and 10 ng/ml LIF. They proliferated for 10 days in culture. The colony appeared to be positive after PAS staining. Claim 13 is included because the cells inherently express SSEA-1 because they are PGCs that are positive after PAS staining.

Claims 1, 2 and 4-14 are rejected under 35 U.S.C. 102(a) as being anticipated by Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539).

Han isolated PGCs from gonads of stage 28 chicken embryos and cultured them on gonadal stromal feeder cells with bovine serum, SCF, LIF, bFGF, IL-11 and IGF for two months (pg 1532, 2.1). The PGCs stained with SSEA-1 (pg 1533, 2.2). The PGCs were injected into the blood vessel of a recipient embryo (pg 1533, 2.3). Four individuals were germline chimeras (pg 1536, 3.3).

Conclusion

No claim is allowed.

Inquiry concerning this communication or earlier communications from the examiner should be directed to Michael C. Wilson who can normally be reached at the office on Monday, Tuesday, Thursday and Friday from 9:30 am to 6:00 pm at 571-272-0738.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

If attempts to reach the examiner are unsuccessful, the examiner's supervisor, Peter Paras, can be reached on 571-272-4517.

The official fax number for this Group is (571) 273-8300.

Michael C. Wilson

MICHAEL WILSON PRIMARY EXAMINER